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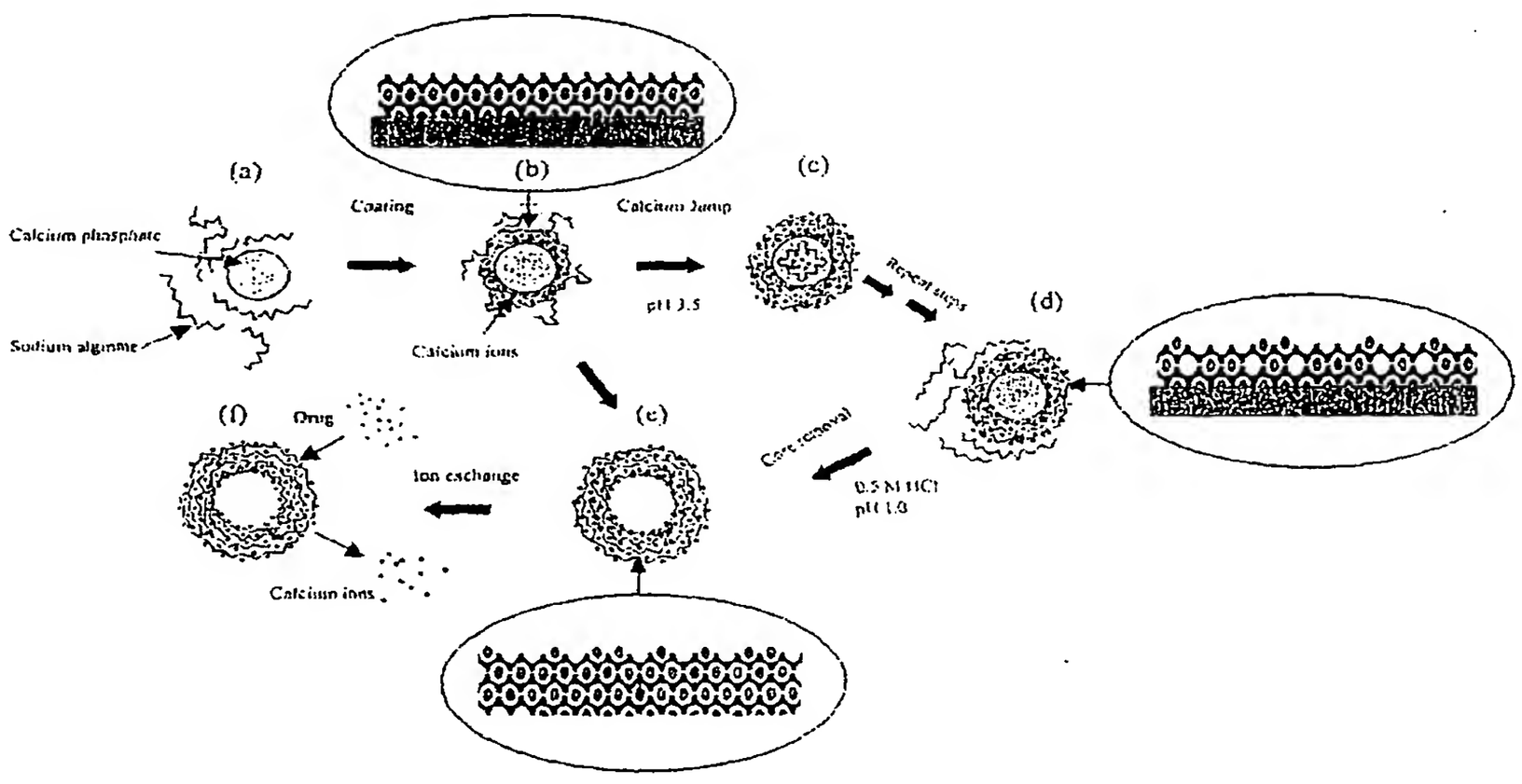
(54) Title: CORE-ASSISTED FORMATION OF MICROCAPSULES



(57) Abstract: The invention refers to a process for producing nanocapsules or/and microcapsules by contacting templates consisting of a core material with a polyelectrolyte.

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Core-Assisted Formation of Microcapsules

Description

The invention refers to a process for producing nanocapsules or/and microcapsules by contacting templates consisting of a core material with a polyelectrolyte.

In recent years many studies have been made on the development of processes for the production of nano- and microcapsules. An important technique which has been developed to produce such capsules is the layer-by-layer (LbL) self-assembly technique (Decher, Science 1997, 277, 1232). The LbL method permits the fabrication of multilayer thin film assemblies on solid supports by spontaneous sequential adsorption of oppositely charged species from dilute aqueous solutions onto charged substrates. The driving force for the multilayer film build-up is primarily due to the electrostatic attraction and complex formation between the charged species deposited.

For example, DE 198 12 083.4, DE 199 07 552.2, EP 98 113 181.6 and WO 99/47252 disclose processes for the production of capsules coated with a polyelectrolyte shell by layer-by-layer application of oppositely charged polyelectrolytes on template particles forming the core of the capsule. An advantage of this process compared to previous processes for the production of microcapsules is that monodisperse capsules with a particularly adjusted wall thickness can be produced.

Applications of such core-shell particles are diverse, ranging from capsule agents for drug delivery, catalysis, coatings, composite materials, as well as for protecting sensitive agents such as enzymes and proteins. An important further development in the field of core-shell particles has been

accomplished by the subsequent removal of the core, resulting in hollow capsules or shells. For the removal of the template core calcining the coated particles at elevated temperature or dissolution of the core material have been described. For example, hollow sub-micron sized shells of yttrium compounds have been produced (Kawahashi and Matijevic, J.Colloid Interface Sci.1991, 143, 103) by coating cationic polystyrene latex with yttrium basic carbonate and subsequently calcining. Further, silica shells were generated by seeded polymerization of tetraethoxysilane on the surface of polystyrene particles, followed by calcination (Bamnolker et al., J.Mater.Sci.Lett.1997, 16, 1412). Using a similar method, monodisperse, hollow silica nanocapsules have been produced by silica coating gold nanoparticles, and by chemically dissolving the cores (Giersig et al., Ber.Bunsenges.Phys.Chem.1997, 101, 1617).

Nano- or microcapsules still including the template core or the hollow shells resulting from the dissolution of the template represent a class of materials which are of great interest in the fields of medicine, pharmaceutics, agriculture and cosmetics. Due to the high requirements concerning uniformity and smoothness of the layer structures, sufficient coverage, control of thickness in combination with the desired stability and permeability, and monodisperse capsule size distribution, there is still need for the development of improved capsule preparation methods.

An object of the present invention therefore was to provide a process for the production of capsules, which is improved compared to known processes.

This problem underlying the present invention is solved by a process for producing nanocapsules or/and microcapsules comprising the steps of

(a) providing templates consisting of a core material comprising positively charged ions, and

(b) contacting the templates with a negatively charged polyelectrolyte,

or the steps of

(a_1) providing templates consisting of a core material comprising negatively charged ions, and

(b_1) contacting the templates with a positively charged polyelectrolyte.

Surprisingly, it has been found that by using the process according to the invention core-assisted formation of self-assembled thin-walled nano- and microcapules can be achieved. The capsules produced in this manner exhibit favorable properties such as adjustable permeability and chemical functionality, biological, chemical and mechanical stability, monodispersity of capsule distribution and adjustable capsule size as required in the field of applications of microcapsules.

The process according to the present invention can either comprise steps (a) and (b) or steps (a_1) and (b_1). In a first embodiment the core material comprises positively charged ions, and the template which consists of this core material is contacted with a negatively charged polyelectrolyte, i.e. a polyelectrolyte that is oppositely charged to the ions comprised by the core material.

In a second embodiment, the core material comprises negatively charged ions and the template consisting of said core material is contacted with a positively charged polyelectrolyte, i.e. again a polyelectrolyte that is oppositely charged to the ions comprised by the core material.

By contacting templates with a polyelectrolyte according to the invention the polyelectrolytes assemble around the templates. Templates that are suitable in the process of this invention consist of a core material comprising components that are able to assist in the formation of

polyelectrolyte layers. Due to their opposite charge compared with the polyelectrolytes the ions comprised in the core material are appropriate for interacting with, binding to or incorporating in the polyelectrolytes surrounding the template. Especially the positively or negatively charged ions in the surface regions of the template can at least partially saturate binding sites on the polyelectrolyte layer, leading to the formation of a stable film. In this way it is possible to provide stable thin-walled nanosized and microsized capsules.

Thus, in a preferred embodiment of the invention, at least part of the core material interacts with, binds to or is incorporated in the polyelectrolytes of the contacting step.

An advantage of the process of the invention is that sterile, biocompatible and biodegradable polyelectrolytes and core materials can be used. Especially in the field of medicine where capsules are practicable for the delivery or/and controlled release of drugs sterile and biocompatible materials are required. Encapsulated active substances such as drugs can be released by controlled degradation of the shell or by permeation through the shell. Thus, it is also preferred to use biodegradable materials that degrade under certains conditions such as, for example, specific pH changes or salt or enzymatic conditions.

Another aspect is that both the polyelectrolyte and the core material can be chosen from recyclable materials. It is possible to recycle the polyelectrolyte and the core material after a simple purification process. In the prior art processes, where oppositely charged polyelectrolytes are used, recycling is not possible because of loss of purity of the polyelectrolyte after the adsorption step and because of the difficult and costly purification process. Thus, by using the process of the present invention, hitherto existing problems such as material loss resulting in high costs can be avoided.

Still another aspect of the process of the invention is that due to the use of predominantly identically charged polyelectrolytes uniform layers with adjustable permeability are formed. Since the core material assists in the formation of the layers and the binding of polyelectrolyte and the core material is strong, the shell also exhibits high biological, chemical and mechanical stability. In contrast thereto, the layer-by-layer method according to the prior art requires the sequential depositing of oppositely charged polyelectrolytes on templates implying the risk of the agglomeration of the polyelectrolytes and consequently the risk of non-uniformity of the layers.

According to the invention preferably > 50% of the polyelectrolytes of the capsule shell have the same charge, more preferably > 70%, even more preferably > 80%, most preferably > 95%.

In particular, by using mainly negatively charged polyelectrolytes according to the process comprising steps (a) and (b) the produced capsules exhibit particularly favorable properties. Since positively charged polyelectrolytes often are toxic, their incorporation in capsules destined, for example, to targeted delivery of drugs through an intravenous route is critical. With this preferred process of the invention it is possible to preferably use solely negatively charged polyelectrolytes and even solely one sort of negatively charged polyelectrolytes, if desired. The resulting one-component polyelectrolyte microcapsules are especially preferred, e.g. if biocompatibility and biodegradability are a major concern. Also in view of the recycling of the capsule materials one-component polyelectrolyte capsules are especially suitable.

In another embodiment of the invention capsules are produced, by applying above all layers with negatively charged polyelectrolytes, wherein, however, positively charged polyelectrolytes are present additionally as one or more intermediate layer(s). In this embodiment the positively charged

polyelectrolytes are preferably deposited in an inner layer of the shell, i.e. the positively charged polyelectrolytes are not present in the surface area of the capsule and are not directly contacting the template.

For certain applications, particularly to offer specific useful property or functionalization to the capsules, it may, however, also be advantageous to provide of positively charged polyelectrolytes in an inner layer and/or an outer layer.

Preferably each of the layers of negatively charged polyelectrolytes in the inner and outer region of the shell (i.e. surface region and region close to the template) independently has a thickness that makes up about 20% of the whole shell thickness, more preferably about 30%, still more preferably about 40%, most preferably about 60%.

In a preferred embodiment the process of the present invention further comprises the step of

(c) at least partially disintegrating the template.

By disintegration of the template ions contained in the core material can get into the surface area of the template and then migrate through the shell. The ions are kept between the polyelectrolytes by interaction and by saturating binding sites on the polyelectrolytes, respectively, and can thus be involved in the build-up of the capsule shell. Consequently, at least part of the core material is incorporated in the shell. This leads to a stabilization of the shell.

The disintegration of the template can be achieved by adjusting the solvent, pH, temperature, salt conditions or by ultrasonic treatment. It is preferred to carry out disintegration for a time period that is sufficient to allow at least partial saturation of the binding sites in the shell. The method

employed for disintegration is chosen depending on the composition of the core material. For example, ethylene diamine, tetraacetic acid or/and other chelating agents can be used for disintegration. Preferably, the disintegration is carried out by at least partially dissolving the core material, i.e. by adding an appropriate solvent, in which the material is soluble or by adjusting the pH such that the core material is at least partially soluble. According to the invention dissolution can be effected in a gentle manner during a short incubation period, e.g. 1 min to 1 h at room temperature. For example, it is practicable to adjust an acidic pH (around pH 2-4), if the core material mainly comprises alkaline earth metal salts.

According to the invention a controlled disintegration of the template is carried out, i.e. the amount of the disintegration can be adjusted. Controlled disintegration preferably is achieved by using e.g. ethylene diamine, tetraacetic acid or/and other chelating agents. In this context partially disintegrating the template denotes that part of the ions are released from the solid core material through which they are made capable of migrating in the polyelectrolyte layers. Thus, some of the core material is not disintegrated and still in a solid or aggregated form. Preferably, ions are released by partially dissolving the core material, but this can also be achieved e.g. by ultrasonic treatment. Preferably, 99% or less of the template are disintegrated, more preferably 95%, still more preferably 80% or less, still more preferably 50% or less and most preferably 25% or less. The minimum degree of disintegration of the template is 0,01%, preferably 1%, more preferably 5%, still more preferably 15%, still more preferably 25% and most preferably 35%.

In a preferred embodiment, step (b) or (b_1) or/and step (c) of the process according to the present invention is/are repeated at least once. By repeating this/these step(s) wall thickness can be controlled. Preferably, the contacting steps and the disintegrating step are repeated such that step (c) is carried out after the contacting step, but any other sequence of

the steps is possible. If desired, only one of the steps is repeated once or several times. Generally, the disintegration step and the contacting step can be carried out simultaneously or subsequently.

During the process of the invention always the same polyelectrolyte can be used or by repeating the contacting step it is possible, for example, to successively use different sorts of polyelectrolytes. These polyelectrolytes can have the same or different charges.

In general, the steps can be repeated as often as necessary to achieve the desired shell thickness or/and capsule size, without having upper limit. Preferably, the step(s) is/are repeated from 2 to 20 times, more preferably from 4 to 15 times, most preferably from 7 to 10 times.

The template according to the present invention can have any suitable form to produce nanocapsules or/and microcapsules. The form may, for example, be spherical, rod-shaped, rectangular, square, triangular and various other forms are possible. The average diameter of the template is 500 μm or less, preferably 50 μm or less and, more preferably, 10 μm or less and, most preferably, 2 μm or less. The minimum diameter of the template is preferably 10 nm, more preferably 100 nm, most preferably 200 nm and, in particular, 1 μm.

The templates consist of a core material which can be crystalline or amorphous. It is also possible to use a single crystal as the template.

The core material is built up by ions, which also comprises core material which is able to form or to set free ions under certain conditions.

The core material of the templates in step (a) comprises positively charged ions which can be selected from positively charged organic substances, inorganic substances or any combination thereof. In a preferred

embodiment the positively charged ions are metal cations. The metal cations can be selected from the group comprising alkaline metal cations, alkaline earth metal cations, cations of main group III metals, transition metal cations and rare earth element cations. Preferred are cations of the metals Al, Ba, Ca, Mg, Y, Tb, Fe, Co, Ni, Cu, Zn and Ag. More preferably, the metal cations are selected from the group comprising Al^{3+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, Y^{3+}, Tb^{3+}, Fe^{2+}, Fe^{3+}, $Co^{2+ \text{ to } 6+}$, $Ni^{2+ \text{ to } 6+}$, $Cu^{2+ \text{ to } 4+}$, Zn^{2+} and Ag^{+}. Also any suitable combination of metal cations is possible. Especially preferred are Ba^{2+}, Ca^{2+} or/and Mg^{2+}.

The core material of the templates in step (a_1) comprises negatively charged ions which can be selected from negatively charged organic substances, inorganic substances or any combination thereof. Preferably, the negatively charged ions are conjugated bases of inorganic or organic acids, in particular, the conjugated bases of inorganic acids. By deprotonating inorganic acids such as water, phosphoric acid, sulfuric acid, nitric acid, carbonic acid, hydrosulfuric acid, sulfurous acid, hydrogen sulfide or hydrohalic acid the respective conjugated bases are formed. In case of polyvalent acids several acid-base pairs are possible accordingly (e.g. in case of H_3PO_4). Preferred conjugated bases of inorganic acids according to the invention are hydroxide, halogenide, nitrate, sulfide, sulfate, carbonate, phosphate, hydrogen phosphate, dihydrogen phosphate and mixtures thereof. Particularly preferred are halogenide, nitrate, sulfate, carbonate and phosphate.

The core material may comprise further components such as organic, inorganic or/and biological substances. These substances can be charged or uncharged. Preferably such substances are selected from the group comprising polymers, drugs, vitamins, nutrients, hormones, growth factors, pesticides, antibiotics, catalysts, preservatives or, in general, an active substance.

For example, also metals, metal oxides or/and organic salts or/and inorganic salts can be incorporated in the core material.

Preferably, in the core material of step (a) suitable counterions of the comprised metal cations are also present. Suitable counterions can be conjugated bases of inorganic or organic acids, preferably the conjugated bases of inorganic acids as defined above.

For the core material of step (a_1) it is preferred that metal cations are additionally present.

In a preferred embodiment of the present invention the core material consists of one or several oxides or/and salts of the metals selected from alkaline earth metals, main group III metals, transition metals and rare earth elements. In particular, the oxides or/and salts of Al, Ba, Ca, Mg, Fe, Co, Ni, Cu or/and Zn are preferred. More particularly, as examples of suitable salts as the core material, the phosphates, sulfates, carbonates, sulfides, hydroxides and halogenides of these metals are used. Especially preferred are $Ba_3(PO_4)_2$, $Ca_3(PO_4)_2$ and $Mg_3(PO_4)_2$.

As metal oxides, for example, CaO, MgO, BaO, Fe_2O_3, Fe_3O_4 and ZnO are preferred.

Preferably, the templates in the process according to the invention are provided as suspension, dispersion or solution in a liquid medium or, if desired, they may also be provided as dry powder. The liquid medium is selected from aqueous solutions, organic solvents and mixtures thereof. Examples of organic solvents are hydrocarbons, alcohols, ethers and esters of carboxylic acids. Preferably pharmaceutically acceptable organic solvents such as dimethyl acetamide, dimethyl sulfoxide, glycols, polyols, N-methyl pyrrolidene and the like can be used.

In step (*b*) of the process according to the present invention the templates are contacted with a negatively charged polyelectrolyte; accordingly, in step (b_1) the templates are contacted with positively charged polyelectrolytes.

Polyelectrolytes are polymers having ionically dissociable groups which may be a component or substituent of the polymer chain. Usually, the number of these ionically dissociable groups in polyelectrolytes is so large that the polymers in dissociated form (also called polyions) are water-soluble. The term polyelectrolytes is understood in this context to cover also ionomers, wherein the concentration of ionic groups is not sufficient for water-solubility, however, which has sufficient charges for undergoing self-assembly. Depending on the kind of dissociable group polyelectrolytes are classified as polyacids, polybases and polyampholytes.

Polybases contain groups which are capable of accepting protons, e.g. by reaction with acids, with a salt being formed. By accepting protons polybases form polycations. Examples of polybases are polyamines such as polyethylene amine, polyvinylamine and polyvinyl pyridine or poly(ammonium salts), such as poly(diallyl dimethylammonium chloride). Polyacids are capable of splitting off protons and thereby forming polyanions. Examples of polyacids are poly(carboxylic acid), polyphosphoric acid, polyvinyl or polystyrene sulphuric acid, polyvinyl or polystyrene sulfonic acid, polyvinyl or polystyrene phosphonic acid and polyacrylic acid. Examples of the respective salts are poly(carboxylate), polyphosphate, polysulphate, polysulfonate, polyphosphonate and polyacrylate.

Polyampholytes contain cationic groups as well as anionic groups.

In the present invention the polyelectrolytes are selected from the group comprising biopolymers, chemically modified biopolymers, synthetic

polymers and mixtures thereof. Also inorganic polymers can be suitable. The polyelectrolytes can be linear or branched, branched polyelectrolytes leading to less compact polyelectrolyte layers having a higher degree of wall porosity. To increase capsule stability polyelectrolyte molecules can be crosslinked within or/and between the individual layers, e.g. by crosslinking amino groups with aldehydes.

Preferred polyelectrolytes in the process of the present invention are biopolymers such as polyamino acids, in particular, peptides, S-layer proteins, lectins, milk protein, antigen proteins, therapeutic proteins such as insulin and calcitonin, polycarbohydrates such as dextrins, pectins, alginates, glycogens, amyloses, chitins or polysialic acids and polynucleotides such as DNA, RNA and oligonucleotides, and chelatins.

Examples of preferred chemically modified biopolymers are carboxymethyl cellulose, carboxymethyl dextran or lignin sulfonates.

Examples of possible inorganic polymers are polysilanes, polysilanoles, polyphosphazenes, polysulfazenes, polysulfides and/or polyphosphates.

According to the present invention it is preferred to use polyelectrolytes which are degradable or biodegradable under certain conditions, e.g. photo-, acid- or base-labile or enzyme labile. With such polyelectrolytes the release of enclosed active substances can be further controlled via the dissolution of the capsule shells. Further, for certain applications, conductive polyelectrolytes or polyelectrolytes having optically active groups can be used as capsule components.

Preferred examples of biodegradable polymers according to the invention are polyglycolic acid (PGA), polylactic acid (PLA), polyamides, poly-2-hydroxy-butyrate (PHB), polycaprolactone (PCL), poly(lactic-co-glycolic) acid (PLGA), and copolymers thereof. Further preferred examples of

polyelectrolytes are labeled polymers, e.g. fluorescence-labeled polymers, conducting polymers, liquid crystal polymers, photoconducting polymers and photochromic polymers as well as copolymers thereof.

Preferred negatively charged polyelectrolytes are e.g. polysialic acid, Gellan gum, alginates, PGA, PLA, PLGA and PHB, and a preferred positively charged polyelectrolyte is poly(diallyl dimethylammonium chloride).

Contacting the templates with a polyelectrolyte according to the invention can be carried out, for example, by first providing a suspension, dispersion or solution of the templates in a liquid medium as defined above and then adding the appropriate polyelectrolytes purely or also in a liquid medium. It is also possible to add the template purely, i.e. as dry powder to polyelectrolytes in a liquid medium. Another possibility is to to add the templates in a liquid medium to the polyelectrolytes in a liquid medium or that both templates and polyelectrolytes are brought together by adding them simultaneously. Thus, contacting the templates and the polyelectrolytes can be carried out in any manner to ensure that the polyelectrolyte molecules can assemble around the templates, leading to templates surrounded by polyelectrolyte molecules.

The process of this invention can be carried out using solely one sort of polyelectrolyte or by using several different polyelectrolytes having the same or different charge.

In a preferred embodiment of the process according to the invention solely negatively charged polyelectrolytes are used.

Still more preferred is the use of solely one sort of negatively charged polyelectrolytes.

According to the process of the invention the polyelectrolyte layers are formed by self-assembly. The respective ions of the core material stabilize the forming shell by interacting or/and binding with or/and incorporation in the arranged polyelectrolytes. If the disintegration step (c) is carried out, the disintegrated core material or the ions, respectively, has/have to pass through the shell and are thereby captured in the polyelectrolyte layer(s). Preferably, the released ions bind with the polyelectrolytes and saturate free binding sites on the polyelectrolytes. The so-formed ion-polyelectrolyte complex model is termed "egg-box complex" due to typical binding sites of ions with the polyelectrolyte resembling an egg-box. Consequently, according to this invention, the core material comprises components suitable for this core-assisted build-up of the shell.

In an especially preferred embodiment of the invention, in step (a) templates are provided which consist of a core material selected from the group consisting of the phosphates, sulfates, carbonates, sulfides, hydroxides or/and halogenides or/and the oxides of Al, Ba, Ca, Mg, Fe, Co, Ni, Cu or/and Zn. Then, in step (b), these templates are contacted with solely negatively charged polyelectrolytes. According to the invention, the metal cations comprised in the core material migrate through the polyelectrolyte surrounding the template and are thereby held between the polyelectrolyte molecules. Through interaction with the polyelectrolytes or by saturating free binding sites on the polyelectrolytes, core-assisted formation of a shell is accomplished. By carrying out also disintegration step (c), even more ions can be incorporated in the shell and, thus, a stable capsule is formed. As mentioned above, all steps of the process can be repeated in any manner. This especially preferred embodiment leads to the formation of a one-component polyelectrolyte capsule having properties that are very useful in the field of applications of micro- and nanocapsules.

In a preferred embodiment of the present invention the process can additionally comprise step

(b_2) removing excess polyelectrolyte, following step (b) or (b_1), respectively.

Preferably, step (b_2) is carried out by washing with pure water or/and a liquid as used in the contacting step. The washing can be done using centrifugation, filtration, decantation, sedimentation or/and a combination of either processes. Step (b_2) is optional and not necessary for carrying out the process of the present invention, but can sometimes be convenient.

In particular, step (b_2) is practicable, if the build-up of the shell is accomplished by repeating the contacting step with a different kind of polyelectrolyte of the same charge or with an oppositely charged polyelectrolyte. Through the washing step excess polyelectrolyte previously used is removed and mixing up of the different polyelectrolytes is avoided. The washing step is particularly preferred, if oppositely charged polyelectrolytes are subsequently used, since these polyelectrolytes may form lumps due to the attraction of the opposite charges. In this case the washing step might be useful to ensure the formation of regular layers leading to a shell exhibiting the required uniformity. Further, it may be helpful to use one or several washing steps (b_2) to remove polyelectrolytes and, thus, control film thickness of the shell.

In another embodiment of the process according to the invention positively or negatively charged ions are additionally added at least once during the process. If positively charged ions are additionally added when performing the process comprising steps (a) and (b) these ions can assist in the desposition of the polyelectrolytes around the template. This may lead to further stabilization of the self-assembled film around the template. Accordingly, additional negatively charged ions can be added when practicing the process comprising steps (a_1) and (b_1).

According to the present invention, adding ions during the process additionally, generally is not required to stabilize the shell around the template, since the core material according to this invention is incorporated in the shell.

In still another preferred embodiment the process of this invention also comprises the step of

(d) completely disintegrating the template.

Preferably, step (d) is carried out after having repeated the contacting or/and disintegrating step (c) as often to having achieved the desired film thickness. However, it is also possible to completely disintegrate the template and then contact the resulting hollow shell with a polyelectrolyte again.

The disintegration is carried out as described above for step (c) except that the conditions are adjusted such that the template is disintegrated completely. This can be achieved e.g. by using ethylene diamine, tetraacetic acid and other chelating agents. In a preferred embodiment an alkaline earth metal salt as the template is dissolved by adjusting a pH of around 1.0. This can be achieved by adding an appropriate acid such as aqueous HCl (e.g. 0.5 M).

In still another embodiment the process of the present invention also comprises the step of

(e) loading the shell or/and the inside of the nanocapsule or/and microcapsule with an active substance.

According to the invention suitable active substances for the loading step are selected from the group comprising reagents, catalysts, enzymes,

pharmaceutical substances and drugs. In a preferred embodiment positively charged drugs such as doxorubicin HCl are loaded in the shell or/and the inside of the capsule.

The loading can be achieved by any suitable process leading to the incorporation of the active substance in the shell and/or the inside of the capsule. This loading is achieved by ionic or hydrophobic interaction, electrostatic interaction, hydrogen bonding, Van-der-Waals interaction or/and a combination thereof. Preferably, the loading is achieved by an ion-exchange process. This means that a charged active substance substitutes an ion of the same charge in the shell or/and the inside, i.e. the hollow space or the template. This can be effected without destroying the shell.

In a particularly preferred embodiment a positively charged drug is loaded in shell and/or the inside by replacing alkaline earth metal cations such as Ba^{2+}, Ca^{2+} or Mg^{2+}, such that the capsule integrity is not damaged.

Another aspect of the present invention is a nanocapsule or/and microcapsule obtainable by the process of the invention.

A capsule according to the invention is defined as being

1. the template surrounded by a shell, i.e. the capsule comprising at least parts of the template provided in step (a) or (a_1)

2. the capsule of 1., the template comprising an active substance

3. the capsule of 1. or 2., wherein the shell or/and the template have been loaded with an active substance according to step (e)

4. the hollow shell, i.e. a capsule, wherein the template has been completely disintegrated

5. the capsule of 4., wherein the shell or/and the hollow space has been loaded with an active substance according to step (e)

6. the capsules of 1. to 5. which have been further chemically modified.

Chemical modification of the capsules can be achieved e.g. by derivatization of free hydroxyl groups or/and unreacted -COOH groups on the shell material.

The capsules according to the invention have an average diameter of 500 μm or less, preferably 50 μm or less, more preferably 10 μm or less, still more preferably 5 μm or less and particularly preferred 2 μm or less. The minimum values of the average diameter of the capsules formed in the described manner is 10 nm, preferably 100 nm, more preferably 200 nm and most preferred 1 μm.

The shell thickness of said capsules can vary in a wide range and is preferably 1,000 nm or less, particularly preferred 500 nm or less, still more preferred 100 nm or less. The minimum values of the shell thickness are preferably 2 nm, more preferably 5 nm and most preferably 30 nm.

The nano- and/or microcapsules obtainable by the process of the invention exhibit properties favorable in the field of applications of nano- and microcapsules. According to the invention monodisperse capsule size distribution is achieved and the produced capsules show considerably higher uniformity and smoothness of the applied layers. In addition thereto, the shells exhibit a desirable biological, chemical and mechanical stability on the one hand, but also, on the other hand, these shells are semi-permeable membrane shells with precisely adjusted permeability. This means the permeability of the membrane is stimuli responsive to stimuli such as solvent, enzyme, photons, pH and ionic concentration. Even if the

template is completely disintegrated, the resulting hollow shell is a free-standing stable capsule. The capsules have an ultrathin shell and, in a preferred embodiment, the templates consist of a core material which is sterile, biocompatible, biodegradable and recyclable. Preferably also the polyelectrolytes used for the build-up of the shell are biocompatible, biodegradable and recyclable.

These properties of the capsules according to the invention make them very useful for targeted delivery or/and controlled release of active substances.

Thus, another aspect of the present invention is the use of a nanocapsule or microcapsule obtainable by the process of the present invention for targeted delivery or/and controlled release of active substances and reagents, and in catalysis.

Preferably, the nanocapsule or microcapsule obtainable by a process of the invention is used for targeted delivery or/and controlled release of positively charged drugs.

Targeting may be accomplished by selecting specific polyelectrolytes and/or core materials for the construction of the capsule which provide specific functional properties allowing targeting of the resulting capsule and also by attaching target specific ligands on the capsule surface. Controlled release of the encapsulated active substances is obtainable by choosing pH swellable, thermally or environmentally responsive and/or biodegradable polyelectrolyte and/or core material. An encapsulated substance also can be released by migrating through the shell. Depending on the active substance to be encapsulated or/and released, the porosity and permeability of the capsule shell can be adjusted.

The use of the capsules according to the invention for targeted delivery and/or controlled release, for example, of drugs may be useful to prevent degradation of the encapsulated material, i.e. the drug, e.g. in the body of animals and humans. The capsules can also be used to control the ability of the encapsulated material to permeate cell walls or bio-membranes. By the nature of the capsule material the resulting capsule can further be used to control the immune reaction of an organism against the encapsulated material. According to the invention the capsules are also able to adhere to mucosal surfaces such as corneal, nasal, pulmonary, oral, vaginal, rectal mucosa, thereby increasing the retention time of the bioactive substance encapsulated therein to provide enhanced efficacy through prolonged action or/and better permeation.

The following Examples and Figures further illustrate the subject matter of the present invention.

Description of the Figures.

Fig.1 is a schematic illustration of the core-assisted polyelectrolyte deposition of a preferred embodiment of the process according to the invention (cf. Example 1).

Fig.2 is a confocal laser scanning image and the corresponding transmission light microscopy image of core-assisted, self-assembled, thin-walled capsules according to the invention loaded with a drug, doxorubicin HCl. The core material used is calcium phosphate.

Example 1

The performing of Example 1 is schematically illustrated in Fig.1.

The initial step involves contacting templates consisting of calcium phosphate with sodium alginate (a). The excess alginate is removed by washing with pure water using centrifugation. The film is formed as a result of binding of alginate with surface calcium (a-b). The pH adjustment disintegrates (dissolves) the core material to produce calcium ions that saturate the binding sites on alginate through "calcium jump" process (c). The excess calcium ions remain on the surface and are used to build the next alginate layer (d). The steps are repeated to control film thickness. Finally, the core is removed by the immediate complete dissolution resulting in capsule formation (e). A positively charged drug is then partially/fully exchanged for calcium ions (f). The calcium-alginate complex is termed egg-box complex due to typical binding site of calcium ion with gluouronic and galactouronic acids of alginate resembling an egg-box. Film formation through egg-box complexation model is illustrated in elliptical magnification.

Claims

1. A process for producing nanocapsules or/and microcapsules comprising the steps of

   (a) providing templates consisting of a core material comprising positively charged ions, and

   (b) contacting the templates with a negatively charged polyelectrolyte,

   or the steps of

   (a_1) providing templates consisting of a core material comprising negatively charged ions, and

   (b_1) contacting the templates with a positively charged polyelectrolyte.

2. The process of claim 1,
wherein at least part of the core material interacts with, binds to or is incorporated in the polyelectrolyte of step (b) or (b_1).

3. The process of claim 1 or 2, also comprising the step of

   (c) at least partially disintegrating the template.

4. The process of one of the preceding claims,
wherein step (b) or (b_1) or/and step (c) is/are repeated at least once.

5. The process of one of the preceding claims, also comprising the step of

   (d) completely disintegrating the template.

6. The process of one of claims 3 to 5,
wherein the disintegration of the template is achieved by adjusting the solvent, pH, temperature, salt conditions or by ultrasonic treatment.

7. The process of one of the preceding claims,
wherein at least part of the core material is incorporated in the shell of the capsules.

8. The process of one of the preceding claims, also comprising step
(b_2) removing excess polyelectrolyte,
following step (b) or (b_1), respectively.

9. The process of claim 8,
wherein step (b_2) is carried out by washing with pure water.

10. The process of one of the preceding claims,
wherein positively or negatively charged ions are additionally added at least once during the process.

11. The process of one of the preceding claims,
wherein the average diameter of the template is 500 μm or less.

12. The process of one of the preceding claims,
wherein the positively charged ions are metal cations.

13. The process of claim 12,
wherein the metal cations are selected from the group comprising alkaline metal cations, alkaline earth metal cations, cations of main group III metals, transition metal cations and rare earth element cations.

14. The process of claim 12 or 13,
wherein the metal cations are selected from the group comprising Al^{3+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, Y^{3+}, Tb^{3+}, Fe^{2+}, Fe^{3+}, $Co^{2+\text{ to }6+}$, $Ni^{2+\text{ to }6+}$, $Cu^{2+\text{ to }4+}$, Zn^{2+} and Ag^{+}.

15. The process of one of the preceding claims,
wherein the negatively charged ions are conjugated bases of inorganic or organic acids.

16. The process of claim 15,
wherein the negatively charged ions are conjugated bases of inorganic acids.

17. The process of claim 16,
wherein the conjugated bases of inorganic acids are selected from the group comprising hydroxide, halogenide, nitrate, sulfide, sulfate, carbonate and phosphate.

18. The process of one of the preceding claims,
wherein the core material consists of one or several oxides or/and salts of the metals selected from alkaline earth metals, group III metals, transition metals and rare earth elements.

19. The process of claim 18,
wherein the core material consists of the phosphates, sulfates, carbonates, sulfides, hydroxides or/and halogenides or/and the oxides of Al, Ba, Ca, Mg, Fe, Co, Ni, Cu or/and Zn.

20. The process of one of the preceding claims,
wherein the polyelectrolyte is selected from the group comprising biopolymers, chemically modified biopolymers and synthetic polymers and mixtures thereof.

21. The process of one of the preceding claims, wherein the polyelectrolyte is a biopolymer.

22. The process of one of the preceding claims, wherein solely one sort of polyelectrolyte is used.

23. The process of one of the preceding claims, wherein solely negatively charged polyelectrolytes are used.

24. The process of one of the preceding claims, wherein solely one sort of negatively charged polyelectrolyte is used.

25. The process of one of the preceding claims, also comprising the step of

(e) loading the shell or/and the inside of the nanocapsule or/and microcapsule with an active substance.

26. The process of claim 25, wherein the active substance is selected from the group comprising reagents, catalysts, enzymes, pharmaceutical substances and drugs.

27. The process of claim 25 or 26, wherein the loading is achieved by an electrostatic interaction, hydropholic interaction or/and a combination of both interactions.

28. The process of claims 25 to 27, wherein the loading is achieved by an ion exchange process.

29. A nanocapsule or/and microcapsule obtainable by the process of one of claims 1 to 28.

30. A nanocapsule or/and microcapsule of claim 29, having an average diameter of 500 μm or less.

31. A nanocapsule or/and microcapsule of claim 29 or 30 with a shell thickness of 100 nm or less.

32. Use of a nanocapsule or microcapsule of one of claims 29 to 31 for targeted delivery or/and controlled release of active substances and reagents, or in catalysis.

33. Use according to claim 32 for targeted delivery or/and controlled release of positively charged drugs.

Fig. 1

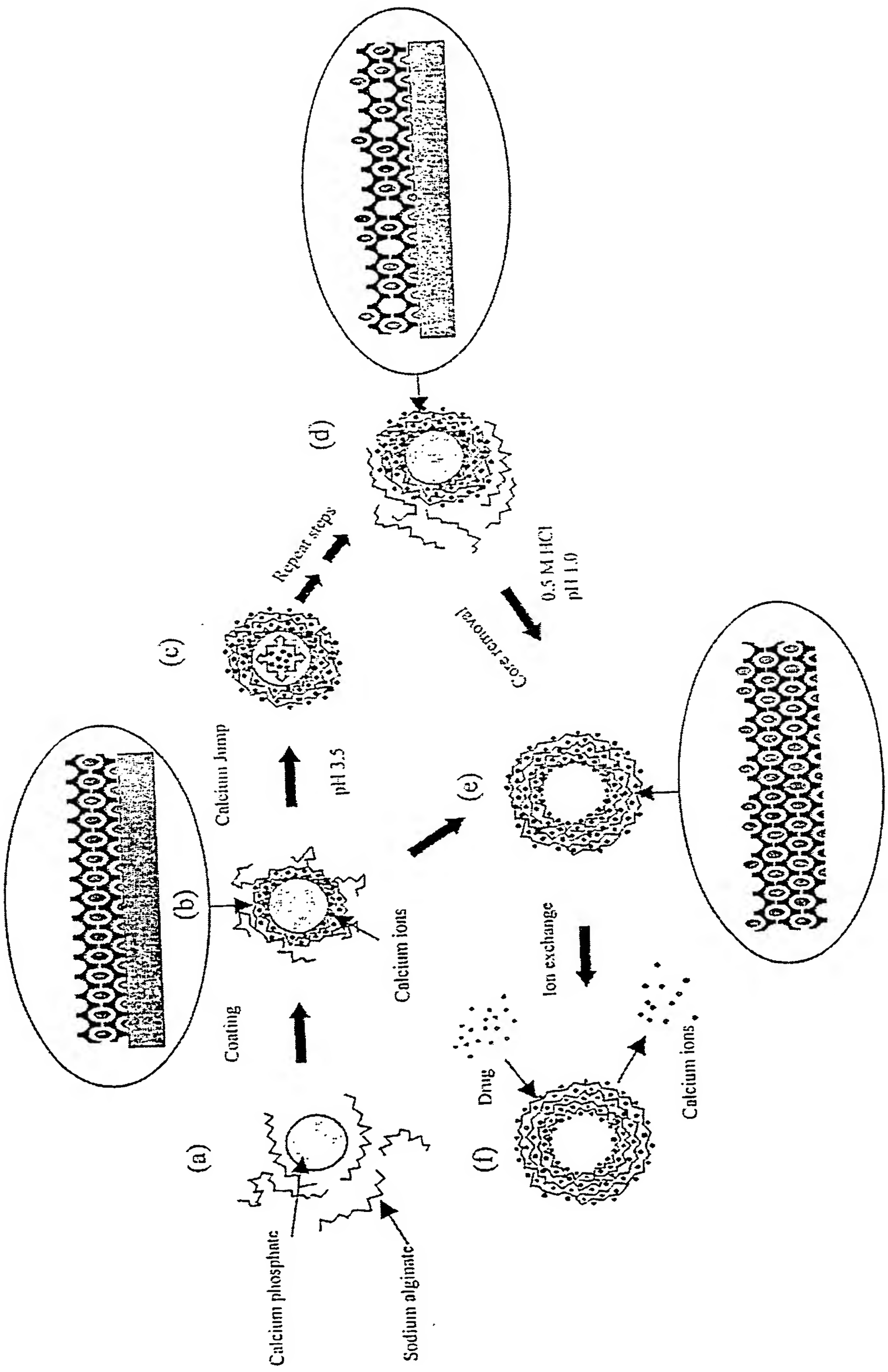
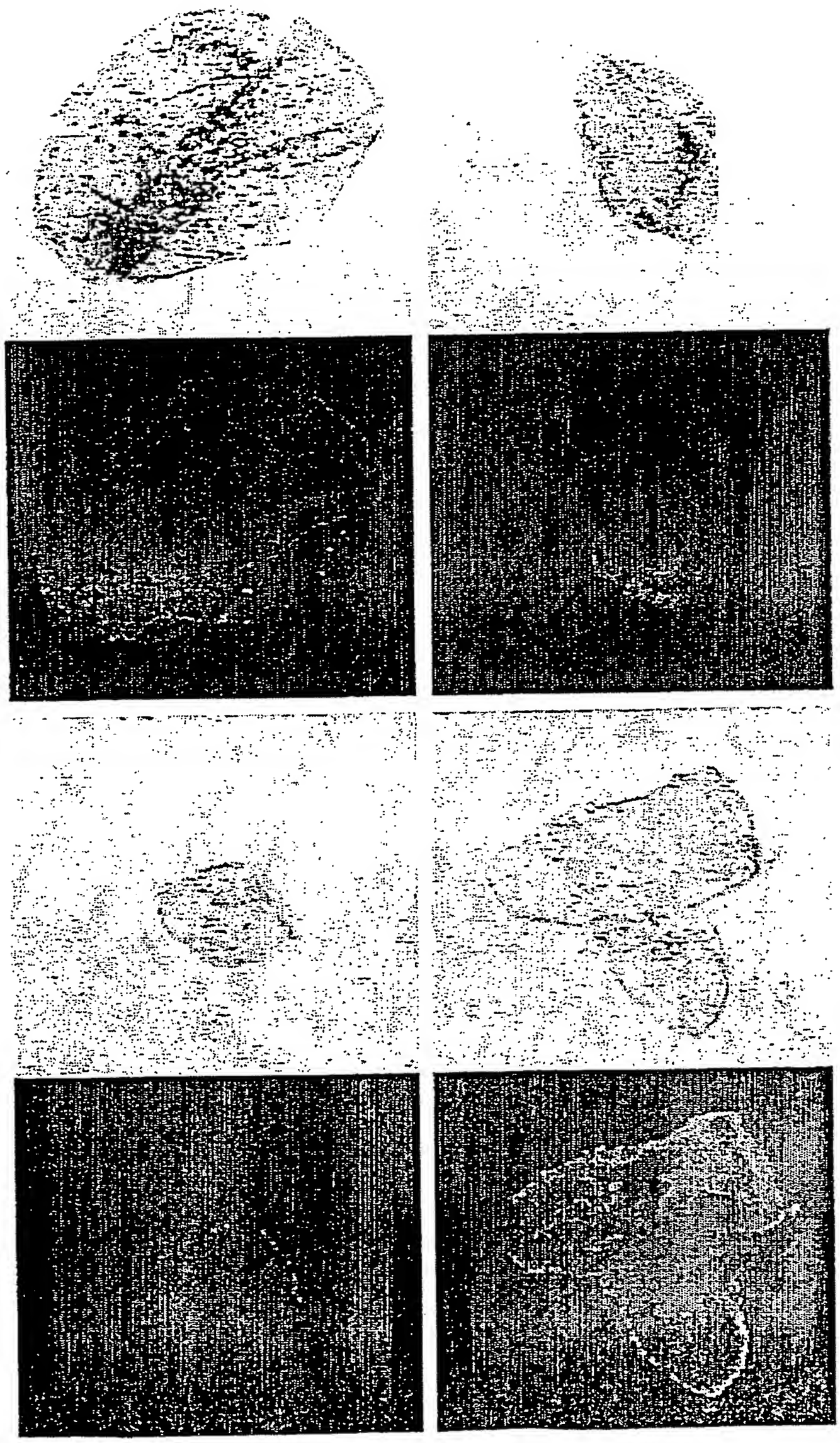

(a)
Calcium phosphate
Sodium alginate
(b)
Coating
(c)
Calcium Jump
pH 3.5
Repeat steps
(d)
Core removal
0.5 M HCl
pH 1.0
(e)
Calcium ions
Ion exchange
(f)
Drug
Calcium ions

Fig. 2



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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/04324

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01J13/02 B01J13/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 77281 A (TRAU DIETER ;RENNEBERG REINHARD (CN); MAX PLANCK GESELLSCHAFT (DE)) 21 December 2000 (2000-12-21) page 4, line 20 -page 15, line 18 page 20, line 1 -page 20, line 21; claims 1-31; figure 1 ---	1-33
X	WO 99 47252 A (LERCHE KARL HEINZ ;MAX PLANCK GESELLSCHAFT (DE); BAEUMLER HANS (DE) 23 September 1999 (1999-09-23) cited in the application page 10, line 15 -page 12, line 2; figure 1 ---	1-33

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 2003	25/07/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Willsher, C

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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